PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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07 NOV 2005

PCT

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 66)

Date of mailing *(day/month/year)*	07.11.2005
Applicant's or agent's file reference DELBE/ P32303PC	**REPLY DUE** **within 2 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/005462	23.12.2004	23.12.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/80, C12N15/67, C12N5/10

Applicant
DELTA BIOTECHNOLOGY LIMITED et al.

1. ☒ The written opinion established by the International Searching Authority:
   ☒ is ☐ is not
   considered to be a written opinion of the International Preliminary Examining Authority

2. This second report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(e).
   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.
   Also: For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4*bis*. For an informal communication with the examiner, see Rule 66.6. For an additional opportunity to submit amendments, see Rule 66.4.

   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 23.04.2006

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WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

International application No.
PCT/GB2004/005462

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1(b))
   ☐ publication of the international application (under Rule 12.4)
   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this opinion is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

   Description, Pages

   1-130 as originally filed

   Claims, Numbers

   1-75 as originally filed

   Drawings, Sheets

   1/63-63/63 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing *(specify)*:
   ☐ any table(s) related to sequence listing *(specify)*:

WRITTEN OPINION OF THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

International application No.
PCT/GB2004/005462

Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-75
	No:	Claims	1
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-75
Industrial applicability (IA)	Yes:	Claims	1-75
	No:	Claims	

2. Citations and explanations:

see separate sheet

In response to a first WRITTEN OPINION of the International Searching Authority, the Applicant has provided arguments (numbered 1-3):

1: *There is no justification for the assertion that chaperones must comprise signal sequences.*
The Applicant has provided examples of cytosolic chaperones affecting secretion of proteins. The argument is persuasive and no restriction to chaperones having signal sequences seems needed. However, as seen below, a restriction of the claimed method to secreted proteins is requested.

2: *D4 provides a prejudice in the art against the expression of cytosolic proteins from a 2 micron plasmid.*
The Applicant argues that D4 shows that 2-micron expression of the cytosolic protein ubiquitin is undesirable compared to chromosomal integration of the expression construct. The Applicant argues that this provides a technical prejudice against 2-micron expression also of cytosolic proteins. However, the present application does not teach successful expression of cytosolic proteins - in particular, there is no data on successful expression of ubiquitin. Thus, even if there were to be a prejudice in the prior art, the present application does not provide a solution to the problem - and consequently, an inventive step can not be motivated with regard to a method for expression of cytosolic proteins from 2-micron plasmids.

3: *D1 fails to provide any motivation to deviate from the teachings of D4 and D3.*
The Applicant questions whether the plasmid used in D1 and specified in D2 is a 2-micron plasmid since "there is no indication that the plasmid encodes FLP, REP1 or REP2". The Applicant is invited to check for the presence of those genes using the available sequence info of pYEX4T-1 found at:
http://orders.clontech.com/clontech/techinfo/vectors_dis/text/pYEX4T-1.txt
Furthermore, the FLP, REP1 or REP2 proteins can also be provided in trans in cir^+ host cells.
In any case, D2 specifies a high copy number plasmid with a 2-micron origin of replication which corresponds to the plasmid defined in claim 1. If the Applicant thinks that a "2 micron family plasmid" corresponds to one containing the genes encoding the FLP, REP1 or REP2 proteins, then this feature should be included in the claims.

The Applicant further states that the method of D1 is qualitative and not quantitative. However, D2 does specify that typical yields obtained with the pYEX4T-1 plasmid are 0.5-6 mg/L. This corresponds to quantitative yields of the fusion protein product.

The Applicant further states that D1 only induces protein expression for 2 hours and does not say anything about long term viability of the culture. However, the present set of claims does not specify any time frames at all. Hence, the issue of the time frame of protein production appears irrelevant.

In summary, the previous inventive step objection is upheld. However, a restriction of the method to production of secreted proteins could potentially be inventive.

Novelty

In addition to the above objections, it appears that at least claim 1 lacks novelty over D1.
Claim 1 specifies:
"A method for producing non-2 micron -family plasmid protein comprising:
(a) providing a host cell comprising a 2 micron-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2 micron-family plasmid protein; (b) culturing the host cell in a culture medium under conditions that allow the expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2 micron-family plasmid protein ; and © purifying the thus expressed non-2 micron-family plasmid protein from the cultured host cell or the culture medium."

Considering that D1 expresses proteins on a genome wide basis, it is apparent that also chaperone proteins are expressed as GST fusions. Thus, in the case of GST-chaperone fusions, the plasmid in D1 will comprise a gene encoding a protein comprising the sequence of a chaperone protein and a gene encoding a non-2 micron family plasmid protein (in this case GST).